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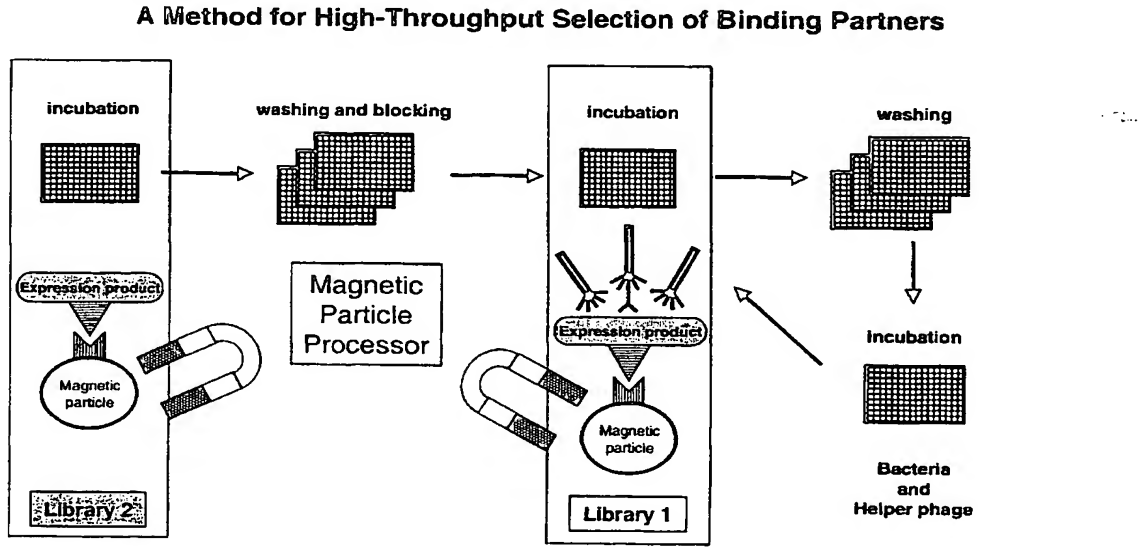

(57) Abstract: The present invention relates to a method for the selection of at least one member of a number of specifically interacting molecules, said method being carried out in (a) container(s), preferably representing an arrayed form, e.g. in (a) microtiter plate(s), using an automated device comprising a magnetic particle processor. In another embodiment, the present invention relates to a method for the production of a pharmaceutical composition comprising the steps of the method of the present invention and further the step of formulating at least one of said specifically interacting molecules selected and/or characterized by the above method or a functionally and/or structurally equivalent derivative thereof in a pharmaceutically acceptable form.

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IPC 7 C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, CAB Data, STRAND, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 57311 A (MAX PLANCK GESELLSCHAFT ;WALTER GERALD (DE); BUESSOW KONRAD (DE);) 11 November 1999 (1999-11-11) claims 1-27	1-6,10, 12-18
P,X	DE 198 54 003 A (JENOPTIK JENA GMBH) 25 May 2000 (2000-05-25) the whole document	1,2

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KALA MRINALINI ET AL: "Magnetic bead enzyme-linked immunosorbent assay (ELISA) detects antigen-specific binding by phage-displayed scFv antibodies that are not detected with conventional ELISA." ANALYTICAL BIOCHEMISTRY, vol. 254, no. 2, 15 December 1997 (1997-12-15), pages 263-266, XP002154707 ISSN: 0003-2697 cited in the application the whole document	
A	MCCONNELL STEPHEN J ET AL: "Biopanning phage display libraries using magnetic beads vs. polystyrene plates." BIOTECHNIQUES, vol. 26, no. 2, February 1999 (1999-02), pages 208-214, XP002154708 ISSN: 0736-6205 cited in the application the whole document	
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A	LUEKING A ET AL: "Protein microarrays for gene expression and antibody screening" ANALYTICAL BIOCHEMISTRY,ACADEMIC PRESS, SAN DIEGO, CA,US, vol. 270, 1999, pages 103-111, XP002127382 ISSN: 0003-2697 cited in the application the whole document	

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BÜSSOW ET AL: "A METHOD FOR GLOBAL PROTEIN EXPRESSION AND ANTIBODY SCREENING ON HIGH-DENSITY FILTERS OF AN ARRAYED cDNA LIBRARY" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 26, no. 21, November 1998 (1998-11), pages 5007-5008, XP002114084 ISSN: 0305-1048 cited in the application the whole document	
T	CHOI J -W ET AL: "A new magnetic bead-based, filterless bio-separator with planar electromagnet surfaces for integrated bio-detection systems" SENSORS AND ACTUATORS B,ELSEVIER SEQUOIA S.A., LAUSANNE,CH, vol. 68, no. 1-3, 25 August 2000 (2000-08-25), pages 34-39, XP004216589 ISSN: 0925-4005 the whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern[illegible]pplication No
PCT/EP 00/06271

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New PCT Patent Application
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METHOD FOR HIGH-THROUGHPUT SELECTION OF INTERACTING MOLECULES

The present invention relates to a method for the selection of at least one member of a number of specifically interacting molecules, said method being carried out in (a) container(s), preferably representing an arrayed form, e.g. in (a) microtiter plate(s), using an automated device comprising a magnetic particle processor. In another embodiment, the present invention relates to a method for the production of a pharmaceutical composition comprising the steps of the method of the present invention and further the step of formulating at least one of said specifically interacting molecules selected and/or characterized by the above method or a functionally and/or structurally equivalent derivative thereof in a pharmaceutically acceptable form.

Cellular functions are controlled by networked expression of gene catalogues. Functional network analysis requires parallel handling of large numbers of gene products and selection and characterization of interacting molecules. For studying protein interactions, two classical library-based approaches are known, an in vitro-method, the so-called yeast Two-Hybrid-System (Mendelsohn & Brent, Science 284, 1999, 1948-1950) and in vitro methods, e.g. phage display. The yeast Two-Hybrid-System has the following disadvantages: (i) transformation efficiency in yeast is low; (ii) protein interactions take place in the milieu of the yeast nucleus and, therefore, interaction parameters can not be controlled; and (iii) only protein-protein interactions are possible to be investigated.
In vitro (e.g. phage surface) display enables the construction of large recombinant peptide and protein libraries for the selection of interacting molecules. The basic concept is a physical link of the phenotype, expressed as g ne product (e.g. displayed on the phage surface) to its coding genetic information (e.g. integrated into the phag

genome). This allows to survey large libraries of organisms (e.g. phage, viruses, bacteria, eukaryotic cells) and/or organelles (e.g. ribosome) and/or soluble molecules (e.g. nucleic acids, protein-nucleic acid hybrids) for the presence of specific molecules using the discriminative power of affinity purification. The selection procedure involves the enrichment of a specific first molecule by binding to an immobilized second (target) molecule. First molecules are enriched by selection (binding and elution) on the target molecule. As a consequence of the physical linkage between genotype and phenotype, sequencing the DNA of the encoding first molecule can readily elucidate the amino acid sequence of the selected gene product.

Peptide libraries were the first libraries to be displayed on phage (Smith, 1985). In the meantime, a wide variety of different peptide libraries were made, with different degrees of randomness and special means of recombination (e.g., Fisch *et al.*, 1996). Peptide libraries are especially useful for mapping interacting parts of proteins (e.g., domains or epitopes). They are also a first step towards the production of small molecules simulating protein actions.

Recombinant immunoglobulin gene libraries cloned in phage or phagemid vectors are an in vitro simulation of antibody repertoires and allow the production of antibodies without immunisation and without the use of animals (reviewed in Winter *et al.*, 1994). Human antibodies against large numbers of different antigens, including human proteins, can be produced by phage selection of single-chain Fv (scFv; Nissim *et al.*, 1994) or Fab fragments (Griffiths *et al.*, 1994). Those antibodies should be particularly valuable as therapeutic agents because the patient's immune system will not recognise them as foreign because they are completely human. Besides antibodies, also enzymes, enzyme inhibitors, receptors, hormones, lymphokines and DNA-binding molecules have been target molecules displayed on filamentous phage. The wide range of possible applications clearly demonstrates the high potential of linking genotype and phenotype as a tool for the development of new molecules.

Although phage display was used extensively for the selection of peptides and antibodies, it had its limitations when it came to the expression of unknown sequences from cDNA libraries. As many of these sequences contain stop codons in their 3' untranslated regions, one cannot directly fuse these sequences to the N-terminus of a

phage coat protein. To overcome this problem, a specialised cloning and expression system has been developed that allows the display of functional cDNA expression products on the surface of filamentous bacteriophage (Crameri & Suter, 1993; Crameri & Blaser, 1995). This system exploits the high-affinity interaction of the Jun and Fos leucine zippers. Gene jun is expressed from a lacZ promoter as a fusion protein with the phage coat protein III. Using a second lacZ promoter of the phagemid pJuFo, gene fos is co-expressed as an N-terminal fusion peptide with the cDNA library gene products, so that the resulting Fos-fusion proteins could become associated with the Jun-decorated phage particles. To avoid inter-phage exchange of fos-cDNA fusion products, cysteines were engineered at the N- and C-termini of each of the leucine zippers, providing a covalent link of the cDNA gene products to the genetic instructions required for their production.

The physical link between phenotype and genotype in phage display allows selective isolation and amplification of a particular phage encoding a desired gene product from pools of millions of phage (Kay *et al.*, 1996). Selection is accomplished by interaction between the displayed gene product and a ligand immobilized on a solid phase. The selected phage are amplified by infection of E. coli cells which, after helper rescue, produce large numbers of new phage. Successive rounds of phage selection and amplification allow selective enrichment of phage displaying gene products with affinity for a desired ligand.

In the prior art, target molecules have been immobilized on plastic surfaces, mainly immunotubes or microtiter plate wells (Harrison *et al.*,1996). Also, magnetic particles have been used for phage display selection (Hawkins *et al.*, 1992, Griffiths *et al.*, 1994, Low *et al.*, 1996, Schier *et al.*, 1996a; Schier *et al.*, 1996b, McConnell *et al.* 1998, McConnell *et al.*, 1999, Kirkham *et al.*, 1999). However, these methods involve the manual handling of the samples and are cumbersome and time-consuming. Accordingly, these methods allow the simultaneous processing of only a very limited number of samples, i.e. they allow only the identification and characterization of one or, at most, a few pairs of interacting molecules per one selection procedure. Moreover, they are difficult to standardize in terms of precisely reproducible conditions.

Thus, the technical problem underlying the present invention was to provide a method that allows the reliable, simultaneous and time-saving high-throughput selection of various members of pairs of interacting molecules.

The solution to the above technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to a method for the selection of at least one member of a number of specifically interacting molecules, said method comprising as the first step involving the contact of said interacting molecules:

(a) contacting a first molecule with a second molecule affixed to a magnetic particle under conditions that allow a specific interaction between said first and second molecule to occur;

and further the steps of:

(b) subjecting the product obtained in step (a) to at least one washing step;

(c) determining whether a specific interaction between said first and second molecule had occurred; and, if said specific interaction had occurred,

(d) providing said first and/or second molecule selected by steps (a) to (c),

wherein steps (a), (b) and (c) are carried out in (a) container(s), preferably representing an arrayed form, e.g. in (a) microtiter plate(s), using an automated device comprising a magnetic particle processor (Fig. 1).

The method of the present invention shows several unexpected advantages in terms of sensitivity, control and automation: First, the number of magnetic particles can be scaled down compared to the manual techniques (e.g. 10-fold to 2 µl or 1.34×10^6 Dynabeads M-280 Streptavidin, Dynal). This causes much less unspecific background binding resulting in a distinct reduction of false positive results.

Second, all washing and incubation conditions can be reproducibly customized. Most importantly, it is envisaged in accordance with the present invention that washing speeds are adjusted to cause different stringencies of selection. This will enable the predictable selection of interacting molecules with different binding affinities. The

washing step which may be repeated at least once in each round of selection is designed to remove first molecules that did not specifically interact with/bind to said second molecules. Appropriate washing conditions can be taken from the appended examples or devised by the person skilled in the art without undue burden.

Third, it is envisaged in accordance with the present invention that the steps of (i) the provision, preferably the recombinant production of said second molecule (that may be a member of one library), (ii) affixing said second molecule to magnetic particles, (iii) optionally blocking of free binding sites on the magnetic particles (to which no second molecules had been affixed), (iv) contacting said first molecule (that may be a member of another library) with said second molecule, (v) washing steps, and (vi) the determination whether a specific interaction between said first and second molecule had occurred, are carried out in (a) container(s) preferably representing an arrayed form, e.g. in (a) microtiter plate(s) or container(s) comprising tubes in an arrayed form, wherein each step is preferably performed in (a) different container(s). The magnetic particle processor comprised in said automated device is used to transfer the magnetic particles between wells of microtiter plates prefilled with the corresponding solutions by capturing the magnetic particles in a first well and releasing the same in a second well of a different microtiter plate, the position of said second well corresponding to the position of said first well. This allows high-throughput selection of interacting molecules as large numbers of, e.g., library clones can be handled in parallel, and the selection of interacting molecules from, e.g., two libraries can be used to create interaction catalogues.

In this regard it is to be understood that a "selection round" comprises steps (a) to (c). Accordingly, the phrase "first step involving the contact of said interacting molecules" denotes the contacting step of the first selection round as compared to second, third, etc. steps involving the contact of said interacting molecules of potential further selection rounds that may be performed subsequently to the first selection round (see below).

A preferred mode of the selection at high-throughput of the invention comprises the following steps: interacting molecules (e.g., anti-protein scFv antibodies) are selected from molecular libraries by a combination of phage display and magnetic bead

technology. Proteins expressed from arrayed cDNA libraries are bound to magnetic beads via a suitable molecular tag (e.g., His_6 or Biotin). Phage displaying specifically interacting molecules are then fished from a library by binding to their interaction partners attached to the beads. This selection involves a sequence of binding and washing steps and was adapted to high throughput using a magnetic particle processor (Labsystems, Helsinki, Finland). Selected molecules are then tested for specificity also employing the magnetic particle processor.

Said first and second molecule may be members of libraries, e.g., an antibody and an antigen library, respectively, i.e. two different libraries. Alternatively, said first and second molecule may be members of the same library. Also comprised by the present invention are embodiments, wherein one molecule (i.e. the first or the second molecule) is a member of a library whereas the other molecule is a compound or a variety of compounds of predetermined specificity. Other options to employ first and second molecules from still different origins or combinations of origins are within the skills of the person skilled in the art.

In a preferred embodiment of the method of the present invention said first and/or second molecule is an organic molecule and/or a mixture of organic and/or inorganic molecules.

In another preferred embodiment, said first and/or second molecule is a hapten.

In a more preferred embodiment of the method of the present invention said first and/or second molecule is a cDNA expression product, and/or a (poly)peptide, and/or a nucleic acid, and/or a lipid, and/or a sugar, and/or a steroid, and/or a hybrid of said molecules.

In a most preferred embodiment said cDNA expression product is an antibody or a fragment or a derivative thereof, an enzyme or a fragment thereof, a surface protein or a fragment thereof, or a nucleic acid-binding protein or a fragment thereof.

Derivatives and fragments of antibodies are well known in the art and comprise, e.g., $F(ab')_2$, Fab, Fv or single chain Fv antibody fragments (see, e.g., Harlow and Lane, "Antibodies, a laboratory manual", CHS Press, 1988, Cold Spring Harbor, N.Y.).

In another preferred embodiment of the method of the present invention said first molecule is a (poly)peptide presented on the surface of organisms (e.g. phage, viruses, bacteria, eukaryotic cells) and/or organelles (e.g. ribosome) and/or soluble molecules (e.g. nucleic acids, protein-nucleic acid hybrids) and the method further comprises after step (b) and prior to step (c) the step of:

(b') amplifying a (poly)peptide specifically interacting with said second molecule,

wherein step (b') is carried out in (a) container(s) preferably representing an arrayed form, e.g. in (a) microtiter plate(s).

In one embodiment of the present invention relating to bacteriophage surface display, the magnetic particle processor may be used to transfer the magnetic particles (to which said bacteriophage is bound via said (poly)peptide specifically interacting with said second molecule) to bacterial culture(s). In another embodiment, the automated device further comprises a shaking device that may be used for shaking the bacterial culture(s) during amplification of said bacteriophages.

It could be demonstrated in accordance with the present invention that after binding of the polypeptide presented on the surface of a bacteriophage to said second (target) molecule affixed to a magnetic particle, said bacteriophage retains its infectivity. Surprisingly, it is possible for the bacteriophage amplification step to infect bacteria directly after a specific interaction has occurred without the need of prior detachment of bound bacteriophage from the magnetic particle.

In a more preferred embodiment of the method of the present invention prior to step (a) said library of first molecules (library 1) is preabsorbed with unloaded magnetic particles and/or molecules competitive (cross-reactive) to second molecules (target, library 2).

For example, if a library of phages is used for performing the method of the invention, this step ensures that phages unspecifically interacting with the magnetic particles are

removed from the phage mixture and only bacteriophages displaying a specifically interacting molecule are selected via this specific interaction. In other words, this step may be performed to further reduce the number of false positive clones.

In an additional more preferred embodiment the method of the present invention further comprises after step (c) and prior to step (d) the step of:

(c') repeating steps (a), (b) and (c) and, optionally, step (b') at least once.

In a most preferred embodiment of the method of the present invention steps (c) and (c') are performed in parallel.

As mentioned above, steps (c) and (c') of the method of the present invention are each carried out in microtiter plates. This advantageously allows the simultaneous performance of steps (c) and (c') in different microtiter plates which further reduces the time required for practicing the method of the present invention.

In a further preferred embodiment of the method of the present invention said number of specifically interacting molecules is a pair of interacting molecules.

In another preferred embodiment of the method of the present invention said number of specifically interacting molecules are three or more interacting molecules.

In yet a further preferred embodiment, the method of the present invention further comprises the step of characterizing said first and/or second molecule and/or the corresponding genetic information.

Methods for the characterization of genetic information, i.e. nucleic acids, and proteinaceous material are well known in the art and include, e.g., nucleic acid sequencing, southern-, northern-, and colony hybridization, primer extension analysis, RNase protection assay, gel shift analysis, western-blotting, ELISA, immunoprecipitation assay, indirect immunofluorescence analysis, and FACS (see, e.g., Sambrook et al., "Molecular cloning - a laboratory manual", Cold Spring Harbor Laboratory (1989) N.Y., Ausubel et al., "Current protocols in molecular biology", Green

Publishing Associates and Wiley Interscience, N.Y. (1989), and Harlow and Lane, loc. cit.).

In another preferred embodiment, said second molecule (target) is affixed to said magnetic particle via an affinity tag (e.g. a metal-chelating tag, an epitope tag, an enzyme binding domain, calmodulin, biotin, Strep-tag, protein A, protein G or protein L) (Fig. 2).

The use of one of the above-mentioned compounds advantageously ensures that said second molecule is affixed to said magnetic particle in a controlled manner and in a predictable orientation, thereby minimally affecting the three-dimensional structure of said second molecule and, consequently, the interacting capacities. Moreover, the use of the above-mentioned compounds allows the direct loading of magnetic particles with second molecules from protein mixtures like, e.g., crude extracts or cell lysates. This is particularly important for high-throughput selection since purification of large numbers of different second molecules is not necessary. However, although the use of the above-mentioned compounds is preferred, the present invention also encompasses the unspecific adsorption of second molecules, e.g., to magnetic particles coated with a plastic surface and/or the covalent binding of second molecules, e.g., via functional groups such as NH_2-, COOH-, SH-groups. These modes of loading may be used especially if partial denaturation and destruction of, e.g., epitopes does not affect the overall efficiency of the method of the present invention.

Moreover, it has been found out in accordance with the present invention that for affixing said second molecule to said magnetic particle preferably saturating concentrations of said second molecule are used so that virtually all free binding sites on said magnetic particle are bound by said second molecule. However, the method of the present invention also encompasses the work with sub-saturating concentrations of said second molecule.

In a most preferred embodiment, said metal-chelating tag is a His-tag, and/or said epitope tag is an HA-tag, a c-myc-tag, a VSV-G-tag, an α-tubulin-tag, a B-tag, an E-tag, FLAG, a His-tag, an HSV-tag, a Pk-tag, a protein C-tag, a T7-tag, EpiTag™, a V5-

tag or an S-tag, and/or said enzyme binding domain is cellulose binding domain, barnase or maltose binding protein.

In an additional preferred embodiment of the method of the present invention step (c) is effected by immunological means.

In a more preferred embodiment of the method of the present invention step (c) is effected by ELISA, RIA, western/colony blotting, FACS or immunohistochemistry.

In another more preferred embodiment of the method of the present invention step (c) is effected in (micro-)array format, preferably on a membrane and/or filter and/or a glas slide and/or in a microtiter plate.

The present invention also relates to a method for the production of a pharmaceutical composition comprising the steps of the method of the present invention and further the step of formulating said first and/or second molecule selected and/or characterized by the method described hereinabove or a functionally and/or structurally equivalent derivative thereof in a pharmaceutically acceptable form.
The pharmaceutical composition of the present invention may comprise a pharmaceutically acceptable carrier and/or diluent. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial administration. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general

health, and other drugs being administered concurrently. A typical dose can be, for example, in the range of 0.001 to 1000 µg (or of nucleic acid for expression or for inhibition of expression in this range); however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. Generally, the regimen as a regular administration of the pharmaceutical composition should be in the range of 1 µg to 10 mg units per day. If the regimen is a continuous infusion, it should also be in the range of 1 µg to 10 mg units per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. Dosages will vary but a preferred dosage for intravenous administration of DNA is from approximately 10^6 to 10^{12} copies of the DNA molecule. The compositions of the invention may be administered locally or systemically. Administration will generally be parenterally, e.g., intravenously; DNA may also be administered directly to the target site, e.g., by biolistic delivery to an internal or external target site or by catheter to a site in an artery. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents such as interleukins or interferons depending on the intended use of the pharmaceutical composition.

The term "functionally and/or structurally equivalent derivative" as used in accordance with the present invention denotes molecules modified, e.g., by deletion, addition and/or substitution of certain parts thereof but essentially maintaining their capacity of specifically interacting with said first or second molecule selected by the method of the present invention. Also encompassed by this term are molecules that have been

modified in order, e.g., to increase their half-lifes in a subject to which they have been administered, to increase the rate of their uptake, to increase their affinity to their interacting counterparts or to increase the excretion rate of the corresponding metabolized end products. With regard to nucleic acids, such molecules may be peptide nucleic acids or nucleic acids comprising, e.g., methylphosphonate- or phosphorothioate-bonds instead of phosphodiester-bonds. Methods for the synthesis of derivatives that, e.g., show the same three-dimensional structure than the originally identified molecule are known in the art and include, e.g., peptidomimetics (see, e.g., Hruby, V.J. et al., Biopolymers 43(3) (1997), 219-66; Bohm, H. J., J. Comput. Aided Mol. Des. 10(4) (1996), 265-272; Wiley, R.A. & Rich, D. H., Med. Res. Rev. 13(3) (1993), 327-384; al-Obeidi, F. et al., Mol. Biotechnol. 9(3) (1998), 205-223; Beeley, N., Trends Biotechnol. 12(6) (1994), 213-6).

Further preferred is to use the compound provided in accordance with the present invention as lead compound for providing downstream developments, in accordance with methods presently employed in the art.

In addition, the invention relates to pharmaceutical compositions comprising at least one of the selected interacting molecules or of derivatives as defined above, optionally in combination with a pharmaceutically acceptable carrier and/or diluent.

The documents cited herein are herewith incorporated by reference.

The figures show:

Figure 1: High-throughput selection of binding partners. Magnetic particles are transferred between wells of microtitre plates, incubated and washed using an automated magnetic particle processor. Molecules of the arrayed Library 2 (targets) are tag-bound to magnetic particles which are washed, blocked and incubated with Library 1 being, e.g., a phage display library. After washing away background phage and incubation

with bacteria and helper phage, an enriched and amplified Library 1 enters the next round of selection against the same Library 2 molecules for further enrichment.

Figure 2: Example for the selection of interacting molecules. Selection from phage display libraries of, e.g. human scFv antibody fragments recognising targets (e.g. specific expression products of a human cDNA library) tag-bound to magnetic particles.

Figure 3: Automated magnetic particle processor (Labsystems, Helsinki, Finland) in action. Left: rod-shaped magnets and plastic caps separated, magnetic particles in solution in microtitre wells; top right: magnets in plastic caps, collection of magnetic particles to plastic caps; bottom right: transfer of magnetic particles to new pre-filled microtitre wells.

Figure 4: Saturation ELISA for assessment of optimal concentrations of protein targets (e.g. bGAPDH) for loading of magnetic particles.

Figure 5: Polyclonal mixtures of phage representing the unselected (rounds) library 1 and the results of every round of selection screened for binding partners to the protein target used for this selection (e.g. UBI8) using magnetic particle ELISA; PTM negative control.

Figure 6: Monoclonal phage (e.g. anti-UBI8) rescreened by magnetic particle phage ELISA for binding to the same protein target (e.g. UBI8); PTM negative control.

The examples illustrate the invention.

Example 1: Automated magnetic particle-handling

An automated device (Fig. 3, Labsystems) was used for washing and incubation of magnetic particles. 96-well microtitre plates (e.g. CliniPlate 200, Labsystems) were pre-filled with solutions (200 µl), and magnetic particles were transferred between wells by capture to and release from rod-shaped magnets covered with plastic caps. Ni-NTA Silica Beads (Qiagen, Hilden, Germany) or Dynabeads M-280 Streptavidin (Dynal, Oslo, Norway) were used for binding of His_6- or biotin-tagged proteins, respectively.

Example 2: Large-scale target production and purification

Protein targets were expressed in *E. coli* (strain SCS1) liquid cultures. 200 ml 2xTY medium (16 g/l Bacto-tryptone, 10 g/l yeast extract, 5 g/l NaCl, pH 7.0) containing 100 µg/ml ampicillin were inoculated with 2 ml of an overnight culture and shaken at 37°C until an OD_{600} of 0.8 was reached. Isopropyl-b-D-thiogalactopyranosid (IPTG) was added to a final concentration of 1 mM. The culture was shaken for 4-6 h at 30 or 37°C. Cells were harvested by centrifugation at 2,100 g for 10 min, resuspended in 5 ml Lysis Buffer (50 mM NaH_2PO_4, 0.3 M NaCl, 10 mM Imidazole, 0.1 mM PMSF, pH 8.0) containing 0.25 mg/ml lysozyme, 10 µg/ml DNase and 10 µg/ml RNase and incubated on ice for 30 min. DNA was sheared with an ultrasonic homogeniser (Sonifier 250, Branson Ultrasonics, Danbury, USA) for 3 x 1 min at 50% power on ice. The lysate was cleared by centrifugation at 10,000 g for 30 min. Ni-NTA agarose (Qiagen) was added and mixed by shaking at 4°C for 1 h. The mixture was poured into a column which was subsequently washed with ten bed volumes of Lysis Buffer containing 20 mM imidazole. Protein was eluted in Lysis Buffer containing 250 mM imidazole and was dialysed against Phosphate-Buffered Saline (PBS, 10 mM Phosphate buffer, 2.7 mM KCl, 137 mM NaCl, pH 7.4) at 4°C overnight.

Proteins were either expressed as fusion proteins with a His_6- and/or biotin-tag attached (Lueking *et al.*, 1999) or were biotinylated in vitro using ImmunoPure NHS-SS-Biotin (Pierce, Rockford USA) and the efficiency of biotinylation was determined by ImmunoPure HABA (Pierce).

Example 3: High-throughput small-scale target production (native conditions)

High-throughput small-scale protein expression and purification was modified according to (Lueking et al., 1999). Briefly, proteins were expressed from selected clones of the arrayed human fetal brain cDNA expression library hEx1 (Büssow *et al.*, 1998). This library was directionally cloned in pQE-30NST for IPTG-inducible expression of His_6-tagged fusion proteins. 96-well microtitre plates (e.g. CliniPlate 200, Labsystems) were filled with 180 µl 2xTY medium supplemented with 100 µg/ml ampicillin. Cultures were inoculated with 20 µl *E. coli* SCS1 cells from overnight cultures. After growth at 37°C with vigorous shaking until an OD_{600} of 0.2 was reached, IPTG was added to a final concentration of 1 mM. Cells were grown for 4-6 h at 30 or 37°C, harvested by centrifugation at 6,000 g for 10 min, washed by resuspension in Lysis Buffer (50 mM NaH_2PO_4, 0.3 M NaCl, 10 mM Imidazole, 0.1 mM PMSF, pH 8.0) containing 0.25 mg/ml lysozyme, 10 µg/ml DNase and 10 µg/ml RNase and incubated on ice for 1 h.

Example 4: High-throughput small-scale target production (denaturing conditions)

High-throughput small-scale protein expression and purification was described (Lueking et al., 1999). Briefly, proteins were expressed from selected clones of the arrayed human fetal brain cDNA expression library hEx1 (Büssow et al., 1998), directionally cloned in pQE-30NST for IPTG-inducible expression of His_6-tagged fusion proteins. 96-well

microtitre plates with 2 ml cavities (StoreBlock, Zinsser) were filled with 100 µl SB medium, supplemented with 100 µg/ml ampicillin and 15 µl/ml kanamycin. Cultures were inoculated with *E. coli* SCS1 cells from 384-well library plates (Genetix, Christchurch, U.K.) that had been stored at -80°C. For inoculation, replicating devices carrying 96 steel pins (length 6 cm) were used. After overnight growth at 37°C with vigorous shaking, 900 µl of prewarmed medium were added to the cultures, and incubation was continued for 1 h. For induction of protein expression, IPTG was added to a final concentration of 1 mM. All following steps, including centrifugations, were also done in 96-well format. Cells were harvested by centrifugation at 1,900 g (3,400 rpm) for 10 min, washed by resuspension in Phosphate Buffer, centrifuged for 5 min and lysed by resuspension in 150 µl Buffer A (6 M Guanidinium-HCl, 0.1 M NaH_2PO_4, 0.01 M Tris-HCl, pH 8.0). Bacterial debris was pelleted by centrifugation at 4,000 rpm for 15 min. Supernatants were filtered through a 96-well filter plate containing a non-protein binding 0.65 µm pore size PVDF membrane (Durapore MADV N 65, Millipore, Bedford, USA) on a vacuum filtration manifold (Multiscreen, Millipore).

Example 5: Magnetic particle loading

Magnetic particles (10 µl or 250 µg Ni-NTA Silica Beads, Qiagen, or 20 µl or 1.34 x 10^7 particles, Dynabeads M-280 Streptavidin, Dynal) were washed twice in PBST (PBS, 0.1 % Tween 20) and loaded with ligands as follows.

(a) Binding from lysates:

Magnetic particles were incubated for 1 h at RT in 200 µl total cell lysate. Examples were proteins like expression products of a human cDNA library as described (Lueking et al., 1999).

(b) Binding of purified targets:

Magnetic particles were incubated for 1 h at RT in 200 µl PBS containing 1% bovine serum albumin (BSA) and the equivalent of 5-10 µM purified targets, depending on the size of target. Examples were proteins, like human glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Swiss-Prot P04406), a C-terminal fragment (40.3 kd) of human heat shock protein 90 alpha (HSP90a, Swiss-Prot P07900), rat immunoglobulin heavy chain binding protein (BIP, Swiss-Prot P06761), tubulin alpha-1 chain (TUBa1, Swiss-Prot P04687), calcium-binding protein ERC-55 precursor (ERC55, Swiss-Prot Q14257), transcription elongation factor S-II (HS-II-T1, Swiss-Prot Q15560), transcription factor ETR101 (ETR101, Swiss-Prot Q03827), peptidyl-prolyl cis-trans isomerase A (EC5218, Swiss-Prot P05092) and Ubiquitin (UBIQ-HUMAN, SWISS-Prot P02248).

After target loading, magnetic particles were washed twice in PBST. Remaining free binding sites were blocked with PTM (PBS, 2% milk powder, 1 % Tween) for 1 h at RT.

Example 6: Magnetic particle ELISA

Magnetic particles (10 µl or 250 µg Ni-NTA Silica Beads, Qiagen, or 2 µl or 1.34 x 10^6 particles, Dynabeads M-280 Streptavidin, Dynal) were incubated with primary and secondary antibodies diluted in PTM for 30 min at RT each and washed twice in PBST after antibody incubations. Secondary antibodies labelled with horseraddish peroxidase (HRP) were detected using ABTS (2,2´-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) solution (10 ml 50 mM Na_3 citrate, 10 ml 50 mM citric acid, 10 mg ABTS, 10 µl 30 % H_2O_2, pH 4.3) and measured as OD 405 nm using SpectraMAX 250 (Molecular Devices, Sunnyvale, USA).

Example 7: Saturation ELISA

Target dilutions (100 nM - 25 µM) were prepared in 200 µl PBS containing 1% BSA and incubated consecutively with two aliquots of magnetic particles (10 µl or 250 µg Ni-NTA Silica Beads, Qiagen, or 20 µl or 1.34 x 10^7 particles, Dynabeads M-280 Streptavidin, Dynal) for 1h at RT each. Magnetic particles were washed twice in PBST, blocked with PTM for 1 h at RT, incubated with primary and secondary antibodies diluted in PTM for 30 min at RT each and washed twice in PBST after antibody incubations. Secondary antibodies labelled with HRP were detected using ABTS solution and measured as OD 405 nm.

Example 8: Phage selection

The preparation of phage from bacterial glycerol stock of phage display libraries was described previously (Harrison *et al.*, 1996). Phage suspensions (10^{12} phage of unselected libraries or the MultiScreen flow-through after phage amplification between selection rounds) were equilibrated and preabsorbed by incubation with unloaded magnetic particles (10 µl or 250 µg Ni-NTA Silica Beads, Qiagen, or 25 µl or 1.68 x 10^7 particles, Dynabeads M-280 Streptavidin, Dynal) in 200 µl PTM for 1h at RT.

Magnetic particles (10 µl or 250 µg Ni-NTA Silica Beads, Qiagen, or 20 µl or 1.34 x 10^7 particles, Dynabeads M-280 Streptavidin, Dynal) were loaded with targets and blocked as described above, incubated with preabsorbed phage for 1 h at RT and washed in PBST several times according to the number of the selection round. *E. coli* TG1 cells were incubated with the washed magnetic particles for 30 min at RT.

Example 9: Phage amplification between selection rounds

20 µl 2xTY containing 10 x Glu-Amp (20% glucose, 1 mg/ml ampicillin) were added and cultures were shaken overnight at 37°C. 10 µl of these

cultures were diluted into 200 µl 2xTY containing 2% glucose, 100 µg/ml ampicillin and shaken at 37°C until OD600 > 0.1 (SpectraMAX 250, Molecular Devices). 10 µl M13-K07 helper phage (10^{12}/ml) were added, cultures were incubated for 30 min at RT and transferred to a Durapore 0.65 µ plate (Millipore). Cultures were sucked through on a MultiScreen vacuum device (Millipore). TG1 cells were resuspended in 200 µl 2xTY containing 100 µg/ml ampicillin and 60 µg/ml kanamycin, transferred to a microtitre plate (e.g. CliniPlate 200, LabSystems) and vigorously shaken overnight at 30°C. Cultures were transferred to a Durapore 0.65 µ plate (Millipore) and sucked through on a MultiScreen vacuum device (Millipore). The flow-through was collected in a microtitre plate (e.g. CliniPlate 200, LabSystems) and was used as starting material (phage suspension) for the next round of selection.

Example 10: Magnetic particle phage ELISA

(a) Polyclonal ELISA

Phage were prepared from overnight cultures as described above. Magnetic particles (10 µl or 250 µg Ni-NTA Silica Beads, Qiagen, or 2 µl or 1.34 x 10^6 particles, Dynabeads M-280 Streptavidin, Dynal) were loaded with targets and blocked as described above, incubated with phage suspensions (10^{10}-10^{11} phage) for 30 min at RT and washed twice in PBST. For detection, magnetic particles were incubated with anti-M13 HRP (1:5,000) in PTM for 30 min at RT, washed twice in PBST and incubated in ABTS solution which was measured at OD 405 nm.

(b) Monoclonal ELISA

The preparation of monoclonal phage was described previously (Harrison et al., 1996). Phage suspensions were divided into two aliquots, diluted 1:1 with PBS and incubated in parallel with magnetic particles either loaded with targets and blocked as described above or unloaded for 30 min at RT. Magnetic particles were washed twice in

PBST, incubated with anti-M13 HRP (1:5,000) in PTM for 30 min at RT, washed twice in PBST and incubated in ABTS solution which was measured at OD 405 nm.

Example 11: PCR and DNA sequencing

PCR and DNA sequencing of antibody genes was described previously (Walter & Tomlinson, 1996).

Example 12: BIAcore analysis

BIAcore analysis of antibody affinity was described previously (Hefta *et al.*, 1996).

Example 13: Specific tag-binding of targets to magnetic particles

His_6- tagged proteins were bound to Ni-NTA Silica Beads (Qiagen), and biotin-tagged proteins were bound to Dynabeads M-280 Streptavidin (Dynal). This tag-binding is specific for the labelled molecules and ensures their proper orientation on the magnetic particles. In contrast, unspecific adsorption of proteins to plastic surfaces leads to partial denaturation and destruction of epitopes. Tag-binding also enables direct loading of magnetic particles with targets from protein mixtures like crude extracts or cell lysates. This is particularly important for high-throughput selection technology, avoiding purification of large numbers of different proteins or other targets.

Example 14: Optimisation of target concentration by Saturation ELISA

The concentration of target molecules on solid surfaces is a critical parameter for the selection of binding partner molecules. Optimal

concentrations of protein targets (e.g. bGAPDH) for loading of magnetic particles were assessed in saturation ELISA experiments (Fig. 4). For most proteins tested, concentrations of 5-10 µM purified target (e.g. 200-400 µg/ml bGAPDH) were found to cause saturation of 10 µl (250 µg) Ni-NTA Silica Beads (Qiagen) or 20 µl (1.34×10^7 particles) Dynabeads M-280 Streptavidin (Dynal). These concentrations are in excess of saturating concentrations of other plastic surfaces (e.g. microtitre plates, Kala *et al.*, 1997) and confirm the high binding capacity of magnetic particles, reflecting their increased surface area. While it is advisable to work with saturating concentrations if possible, some protein targets can not be produced in sufficient amounts. In such cases, sub-saturating concentrations of protein targets were used successfully, due to the high sensitivity of the magnetic particle ELISA (data not shown).

Example 15: High-throughput selection and screening of binding partners

Phage display libraries (e.g. human scFv antibody fragment libraries, Tomlinson, unpublished) were screened for binders to various protein targets (see above). Phage titres were recorded after each round of selection to monitor the efficiency of phage amplification (data not shown). Using the magnetic particle phage ELISA, polyclonal mixtures of phage representing the unselected library and the results of every round of selection were screened for binding partners to the protein target used for this selection (e.g. UBI8, Fig. 5). Positive mixtures were cloned, and single colonies were rescreened by magnetic particle phage ELISA for binding to the same protein target (e.g. UBI8, Fig. 6).

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CLAIMS

1. A method for the selection of at least one member of a number of specifically interacting molecules, said method comprising as the first step involving the contact of said interacting molecules:

   (a) contacting a first molecule with a second molecule affixed to a magnetic particle under conditions that allow a specific interaction between said first and second molecule to occur;

   and further the steps of:

   (b) subjecting the product obtained in step (a) to at least one washing step;

   (c) determining whether a specific interaction between said first and second molecule had occurred; and, if said specific interaction had occurred,

   (d) providing said first and/or second molecule selected by steps (a) to (c),

   wherein steps (a), (b) and (c) are carried out in (a) container(s) preferably representing an arrayed form, e.g. in (a) microtiter plate(s), using an automated device comprising a magnetic particle processor.

2. The method of claim 1, wherein said first and/or second molecule is an organic molecule and/or a mixture of organic molecules and/or inorganic molecules.

3. The method of claim 1 or 2, wherein said first and/or second molecule is a hapten.

4. The method of claim 2 or 3, wherein said first and/or second molecule is a cDNA expression product, and/or a (poly)peptide, and/or a nucleic acid, and/or a lipid, and/or a sugar, and/or a steroid, and/or a hybrid of said molecules.

5. The method of claim 4, wherein said cDNA expression product is an antibody or a fragment or a derivative thereof, an enzyme or a fragment thereof, a surface protein or a fragment thereof, or a nucleic acid-binding protein or a fragment thereof.

6. The method of any one of claims 1 to 5, wherein said first molecule is a (poly)peptide presented on the surface of organisms (e.g. phage, viruses, bacteria, eukaryotic cells) and/or organelles (e.g. ribosome) and/or soluble molecules (e.g. nucleic acids, protein-nucleic acid hybrids) and wherein the method further comprises after step (b) and prior to step (c) the step of:
(b') amplifying a (poly)peptide specifically interacting with said second molecule, wherein step (b') is carried out in (a) container(s) preferably representing an arrayed form, e.g. in (a) microtiter plate(s).

7. The method of claim 6, wherein prior to step (a) said library of first molecules (library 1) is preabsorbed with unloaded magnetic particles and/or molecules competitive (cross-reactive) to second molecules (target, library 2).

8. The method of claim 6 or 7 which further comprises after step (c) and prior to step (d) the step of:
(c') repeating steps (a), (b) and (c) and, optionally, step (b') at least once.

9. The method of claim 8, wherein steps (c) and (c') are performed in parallel.

10. The method of any one of claims 1 to 9, wherein said number of specifically interacting molecules is a pair of interacting molecules.

11. The method of any one of claims 1 to 9, wherein said number of specifically interacting molecules are three or more interacting molecules.

12. The method of any one of claims 1 to 11 further comprising the step of characterizing said first and/or second molecule and/or the corresponding genetic information.

13. The method of any one of claims 1 to 12, wherein said second molecule target is affixed to said magnetic particle via an affinity tag (e.g. a metal-chelating tag, an epitope tag, an enzyme binding domain, calmodulin, biotin, Strep-tag, protein A, protein G or protein L) and/or unspecific adsorption (e.g. plastic surface) and/or covalent binding (e.g. via functional groups such as NH_2-, COOH-, SH-groups).

14. The method of claim 13, wherein said metal-chelating tag is a His-tag, and/or said epitope tag is an HA-tag, a c-myc-tag, a VSV-G-tag, an α-tubulin-tag, a B-tag, an E-tag, FLAG, a His-tag, an HSV-tag, a Pk-tag, a protein C-tag, a T7-tag, EpiTag™, a V5-tag or an S-tag, and/or said enzyme binding domain is cellulose binding domain, barnase or maltose binding protein.

15. The method of any one of claims 1 to 14, wherein step (c) is effected by immunological means.

16. The method of claim 15, wherein step (c) is effected by ELISA, RIA, western/colony blotting, FACS or immunohistochemistry.

17. The method of claim 15 or 16, wherein step (c) is effected in (micro-)array format, preferably on a membrane and/or filter and/or a glas slide and/or in a microtiter plate.

18. A method for the production of a pharmaceutical composition comprising the steps of the method of any one of claims 1 to 17 and further the step of formulating said first and/or second molecule selected and/or characterized by the method of any one of claims 1 to 17 or a functionally and/or structurally equivalent derivative thereof in a pharmaceutically acceptable form.

SUMMARY

The present invention relates to a method for the selection of at least one member of a number of specifically interacting molecules, said method being carried out in (a) container(s), preferably representing an arrayed form, e.g. in (a) microtiter plate(s), using an automated device comprising a magnetic particle processor. In another embodiment, the present invention relates to a method for the production of a pharmaceutical composition comprising the steps of the method of the present invention and further the step of formulating at least one of said specifically interacting molecules selected and/or characterized by the above method or a functionally and/or structurally equivalent derivative thereof in a pharmaceutically acceptable form.

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Date of mailing (day/month/year) 25 April 2001 (25.04.01)	
International application No. PCT/EP00/06271	**Applicant's or agent's file reference** D 1920 PCT
International filing date (day/month/year) 04 July 2000 (04.07.00)	**Priority date** (day/month/year) 05 July 1999 (05.07.99)
Applicant WALTER, Gerald et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

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Date of mailing *(day/month/year)*	17.10.2001
Applicant's or agent's file reference D 1920 PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/06271	04/07/2000	05/07/1999

Applicant
MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER ...

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

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International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/06271	04/07/2000	05/07/1999
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1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

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   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24/01/2001	17.10.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Valcarcel, R Telephone No. +49 89 2399 2368

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/06271

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-24	as originally filed

Claims, No.:

1-18	as received on	24/09/2001	with letter of	24/09/2001

Drawings, sheets:

1/6-6/6	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
see separate sheet

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18
	No:	Claims	NONE
Inventive step (IS)	Yes:	Claims	NONE
	No:	Claims	1-18

Industrial applicability (IA) Yes: Claims 1-18
No: Claims NONE

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
s e separate sheet

Re Item I

The amendments filed with the letter dated 24.09.2001 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendment concerned is the one introduced in claim 1 (d): "wherein steps (a), (b) and (c) are carried out **in parallel**. On page 5 of the description it is stated that library clones can be handled in parallel. The IPEA considers that this is not sufficient basis for the amended claim 1(d), since it is not disclosed that the steps (a), (b) and (c) are to be carried out in parallel. Nowhere else in the application as filed was found a basis for the cited amendment. Thus, the cited amendment has not been considered in this report and originally filed claim 1(d) has been considered instead.

Re Item IV

The present application lacks unity contravening Rule 13 PCT. The IPEA cannot find any "special technical feature" (in the sense of Rule 13.2 PCT) which links the different methods referred to in claims 1 to 18 of the present application. However, the IPEA elected to carry out examination for the subject-matter of all claims.

Re Item V

1. The document numbering corresponds to the order of citation in the search report.

2. The following documents (D) were not cited in the international search report.
   D10: DE 29614623 U
   D11: WO 9325912 A

3. The present application does not meet the requirements of Article 33.3 PCT since **the subject-matter of claim 1 does not involve an inventive step.**

   D3 and **D4** disclose methods for selection of at least one molecule able to interact with a given molecule comprising the steps of:
   (a) contacting a first molecule with a second molecule affixed to a magnetic particle (in D3 the heat-stable alkaline phosphatase, see abstract; and in D4 anti-IL-8, see first page, third column) under conditions allowing specific interaction:
   (b) subjecting the product of step (a) to at least one washing step (PBS in D3, see page 265, left column, second paragraph; Tris-buffered saline plus Tween 20 in D4, see second page, middle column);

(c) determining whether a specific interaction had occurred (see in D3 page 265, left column, second paragraph; and see second and third pages of D4);
(d) providing the molecules selected by steps (a) to (c) (the positive molecules were isolated in D3 and D4 for further analysis);
wherein steps (a) to (c) are carried in containers (microcentrifuge tubes or ELISA plates in D3; polypropylene centrifuge tubes in D4) using a magnetic particle separator (in D3 a magnetic separator from SIGMA, see page 264, right column, first paragraph; in D4 it is not specified which magnetic separator was used but it is assumed that one was used).

Thus, D3 and D4 disclose methods having all the technical features and technical effects referred to in claim 1, with the exception of the use of an **"automated device comprising a magnetic particle processor"**. The technical problem would have been to provide a method using an alternative magnetic separator. The solution of the present application is the use of an automated device comprising a magnetic particle processor. The solution does not involve an inventive step for the following reasons.

D10 and **D11** disclose automated devices comprising a magnetic particle processor. The person skilled in the art would have combined the teachings of either D3 or D4, with the teachings of D10 and D11 to arrive at the same solution as the one disclosed in claim 1 of the present application, since the advantages of using the devices of D10 or D11 in the methods of D3 and D4 are obvious. **Thus, the subject-matter of claim 1 does not involve an inventive step.**

It is noted that D3 focuses on the improvement of detection of a library of phage-displayed antibodies against one specific antigen (the heat-stable alkaline phosphatase). It is further noted that D4 compares the efficiency of biopanning phage display libraries by traditional plate vs. magnetic bead methods using a monoclonal antibody to human IL-8. However, the methods disclosed in D3 and D4 are applicable to a variety of different compounds. The fact that the experiments disclosed in D3 and D4 have been performed to screen display libraries by using either a phosphatase (D3) or an antibody (D4), does not mean that the skilled person would not contemplate to use also a variety of compounds (including a library of antigens, or a library of antibodies) to screen against the display libraries of D3 and

D4. The skilled person would contemplate this possibility taking into account the standard knowledge in the field, and the obvious advantage of screening simultaneously different compounds.

Furthermore, D4 discloses that the fact that biopanning using magnetic beads is more effective than plate biopanning has several practical consequences for investigators attempting to isolate phage capable of binding to biological targets (see page 214, middle column, second paragraph). Such biological targets comprise a vast variety of possible compounds. Thus, the IPEA considers that the skilled person would contemplate to use of automated devices (as the ones disclosed in D10 and D11) together with the methods of selection disclosed in either D3 or D4 to arrive at the same solution as the one proposed in claim 1 of the present application.

Furthermore, the skilled person would also contemplate to carry out the different steps using an array-based technology in view of the obvious advantage of screening simultaneously a plurality of compounds against a library of potentially interacting compounds.

4. Claims 2 to 18 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, and thus **the subject-matter of claims 2 to 18 does also not involve an inventive step.**

Re Item VI Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 99 57311	11 November 1999	30 April 1999	30 April 1998
DE 198 54 003	25 May 2000	18 November 1998	18 Nov.1998

Re Item VIII

Claim 1 is unclear (and therefore also claims 2 to 18 which refer to it) since the expression **"specifically interacting molecules"** is vague and undefined rendering the scope of these claims unclear. Furthermore, the expression **"magnetic particle processor"** is also unclear form the wording of the claim alone in contrast to the requirements of Article 6 PCT (see PCT Preliminary Examination Guidelines, Chapter III, Section 4.2).

CLAIMS

1. A method for the selection of at least one member of a number of specifically interacting molecules from libraries, said method comprising as the first step involving the contact of said interacting molecules:

   (a) contacting a first molecule with a second molecule affixed to a magnetic particle under conditions that allow a specific interaction between said first and second molecule to occur;

   and further the steps of:

   (b) subjecting the product obtained in step (a) to at least one washing step;

   (c) determining whether a specific interaction between said first and second molecule had occurred; and, if said specific interaction had occurred,

   (d) providing said first and/or second molecule selected by steps (a) to (c),

   wherein steps (a), (b) and (c) are carried out in parallel in (a) container(s) preferably representing an arrayed form, e.g. in (a) microtiter plate(s), using an automated device comprising a magnetic particle processor.

2. The method of claim 1, wherein said first and/or second molecule is an organic molecule and/or a mixture of organic molecules and/or inorganic molecules.

3. The method of claim 1 or 2, wherein said first and/or second molecule is a hapten.

4. The method of claim 2 or 3, wherein said first and/or second molecule is a cDNA expression product, and/or a (poly)peptide, and/or a nucleic acid, and/or a lipid, and/or a sugar, and/or a steroid, and/or a hybrid of said molecules.

5. The method of claim 4, wherein said cDNA expression product is an antibody or a fragment or a derivative thereof, an enzyme or a fragment

thereof, a surface protein or a fragment thereof, or a nucleic acid-binding protein or a fragment thereof.

6. The method of any one of claims 1 to 5, wherein said first molecule is a (poly)peptide presented on the surface of organisms (e.g. phage, viruses, bacteria, eukaryotic cells) and/or organelles (e.g. ribosome) and/or soluble molecules (e.g. nucleic acids, protein-nucleic acid hybrids) and wherein the method further comprises after step (b) and prior to step (c) the step of:

(b') amplifying a (poly)peptide specifically interacting with said second molecule,

wherein step (b') is carried out in (a) container(s) preferably representing an arrayed form, e.g. in (a) microtiter plate(s).

7. The method of claim 6, wherein prior to step (a) said library of first molecules (library 1) is preabsorbed with unloaded magnetic particles and/or molecules competitive (cross-reactive) to second molecules (target, library 2).

8. The method of claim 6 or 7 which further comprises after step (c) and prior to step (d) the step of:

(c') repeating steps (a), (b) and (c) and, optionally, step (b') at least once.

9. The method of claim 8, wherein steps (c) and (c') are performed in parallel.

10. The method of any one of claims 1 to 9, wherein said number of specifically interacting molecules is a pair of interacting molecules.

11. The method of any one of claims 1 to 9, wherein said number of specifically interacting molecules are three or more interacting molecules.

12. The method of any one of claims 1 to 11 further comprising the step of characterizing said first and/or second molecule and/or the corresponding genetic information.

13. The method of any one of claims 1 to 12, wherein said second molecule target is affixed to said magnetic particle via an affinity tag (e.g. a metal-chelating tag, an epitope tag, an enzyme binding domain, calmodulin, biotin, Strep-tag, protein A, protein G or protein L) and/or unspecific adsorption (e.g. plastic surface) and/or covalent binding (e.g. via functional groups such as NH_2-, COOH-, SH-groups).

14. The method of claim 13, wherein said metal-chelating tag is a His-tag, and/or said epitope tag is an HA-tag, a c-myc-tag, a VSV-G-tag, an α-tubulin-tag, a B-tag, an E-tag, FLAG, a His-tag, an HSV-tag, a Pk-tag, a protein C-tag, a T7-tag, EpiTag™, a V5-tag or an S-tag, and/or said enzyme binding domain is cellulose binding domain, barnase or maltose binding protein.

15. The method of any one of claims 1 to 14, wherein step (c) is effected by immunological means.

16. The method of claim 15, wherein step (c) is effected by ELISA, RIA, western/colony blotting, FACS or immunohistochemistry.

17. The method of claim 15 or 16, wherein step (c) is effected in (micro-)array format, preferably on a membrane and/or filter and/or a glas slide and/or in a microtiter plate.

18. A method for the production of a pharmaceutical composition comprising the steps of the method of any one of claims 1 to 17 and further the step of formulating said first and/or second molecule selected and/or characterized by the method of any one of claims 1 to 17 or a functionally and/or structurally equivalent derivative thereof in a pharmaceutically acceptable form.

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
VOSSIUS & PARTNER
Siebertstrasse 4
D-81675 München
ALLEMAGNE

EINGEGANGEN
Vossius & Partner
25. Mai 2001
Frist
bearb.:

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	23.05.2001
REPLY DUE	**within 3 month(s)** from the above date of mailing

Applicant's or agent's file reference	
D 1920 PCT	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/06271	04/07/2000	05/07/1999

International Patent Classification (IPC) or both national classification and IPC

C12N15/10

Applicant

MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER ...

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☒	Certain document cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 05/11/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Valcarcel, R

Formalities officer (incl. extension of time limits)
Büchler, S
Telephone No. +49 89 2399 8090

I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement *sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

Description, pages:

1-24 as originally filed

Claims, No.:

1-18 as originally filed

Drawings, sheets:

1/6-6/6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:
see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	
Inventive step (IS)	Claims	1-18 (NO)
Industrial applicability (IA)	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item IV

The present application lacks unity contravening Rule 13 PCT. The IPEA cannot find any "special technical feature" (in the sense of Rule 13.2 PCT) which links the different methods referred to in claims 1 to 18 of the present application. However, the IPEA has elected to carry out examination for the subject-matter of all claims.

Re Item V

1. The document numbering corresponds to the order of citation in the search report.

2. The following documents (D) were not cited in the international search report. Copies of the documents are appended hereto.
   D10: DE 29614623 U
   D11: WO 9325912 A

3. The present application does not meet the requirements of Article 33.3 PCT since **the subject-matter of claim 1 does not involve an inventive step.**

   D3 and **D4** disclose methods for selection of at least one molecule able to interact with a given molecule comprising the steps of:
   (a) contacting a first molecule with a second molecule affixed to a magnetic particle (in D3 the heat-stable alkaline phosphatase, see abstract; and in D4 anti-IL-8, see first page, third column) under conditions allowing specific interaction:
   (b) subjecting the product of step (a) to at least one washing step (PBS in D3, see page 265, left column, second paragraph; Tris-buffered saline plus Tween 20 in D4, see second page, middle column);
   (c) determining whether a specific interaction had occurred (see in D3 page 265, left column, second paragraph; and see second and third pages of D4);
   (d) providing the molecules selected by steps (a) to (c) (the positive molecules were isolated in D3 and D4 for further analysis);
   wherein steps (a) to (c) are carried in containers (microcentrifuge tubes or ELISA plates in D3; polypropylene centrifuge tubes in D4) using a magnetic particle separator (in D3 a magnetic separator from SIGMA, see page 264, right column, first paragraph; in D4 it is not specified which magnetic separator was used but it is assumed that one was used).

Thus, D3 and D4 disclose methods having all the technical features and technical effects referred to in claim 1, with the exception of the use of an **"automated device comprising a magnetic particle processor"**. The technical problem would have been to provide a method using an alternative magnetic separator. The solution of the present application is the use of an automated device comprising a magnetic particle processor. The solution does not involve an inventive step for the following reasons.

D10 and **D11** disclose automated devices comprising a magnetic particle processor. The person skilled in the art would have combined the teachings of either D3 or D4, with the teachings of D10 and D11 to arrive at the same solution as the one disclosed in claim 1 of the present application, since the advantages of using the devices of D10 or D11 in the methods of D3 and D4 are obvious. **Thus, the subject-matter of claim 1 does not involve an inventive step.**

4. Claims 2 to 18 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, and thus **the subject-matter of claims 2 to 18 does also not involve an inventive step.**

Re Item VI Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 99 57311	11 November 1999	30 April 1999	30 April 1998
DE 198 54 003	25 May 2000	18 November 1998	18 Nov.1998

Re Item VIII

Claim 1 is unclear (and therefore also claims 2 to 18 which refer to it) since the expression **"specifically interacting molecules"** is vague and undefined rendering the scope of these claims unclear. Furthermore, the expression **"magnetic particle processor"** is also unclear form the wording of the claim alone in contrast to the requirements of Article 6 PCT (see PCT Preliminary Examination Guidelines, Chapter III, Section 4.2).

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
VOSSIUS & PARTNER
Siebertstrasse 4
D-81675 München
GERMANY

EINGEGANGEN
Vossius & Partner
27. Dez. 2000
Frist heard..

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 22/12/2000
Applicant's or agent's file reference D 1920 PCT	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/EP 00/ 06271	International filing date *(day/month/year)* 04/07/2000
Applicant MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER ...	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mireille Claudepierre

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

BNSDOCID: <XSISA220NOENP4_I_>

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

BNSDOCID: <XSISA220NOENP4_1_>

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
D 1920 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 06271	04/07/2000	05/07/1999
Applicant		
MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER ...		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/06271

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 C12N15/62 G01N33/543 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, CAB Data, STRAND, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 57311 A (MAX PLANCK GESELLSCHAFT ;WALTER GERALD (DE); BUESSOW KONRAD (DE);) 11 November 1999 (1999-11-11) claims 1-27 ---	1-6,10, 12-18
P,X	DE 198 54 003 A (JENOPTIK JENA GMBH) 25 May 2000 (2000-05-25) the whole document --- -/--	1,2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 December 2000	22/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KALA MRINALINI ET AL: "Magnetic bead enzyme-linked immunosorbent assay (ELISA) detects antigen-specific binding by phage-displayed scFv antibodies that are not detected with conventional ELISA." ANALYTICAL BIOCHEMISTRY, vol. 254, no. 2, 15 December 1997 (1997-12-15), pages 263-266, XP002154707 ISSN: 0003-2697 cited in the application the whole document ---	
A	MCCONNELL STEPHEN J ET AL: "Biopanning phage display libraries using magnetic beads vs. polystyrene plates." BIOTECHNIQUES, vol. 26, no. 2, February 1999 (1999-02), pages 208-214, XP002154708 ISSN: 0736-6205 cited in the application the whole document ---	
A	MAIER ET AL: "AUTOMATED ARRAY TECHNOLOGIES FOR GENE EXPRESSION PROFILING" DRUG DISCOVERY TODAY,GB,ELSEVIER SCIENCE LTD, vol. 2, no. 8, August 1997 (1997-08), pages 315-324, XP002103832 ISSN: 1359-6446 the whole document ---	
A	LEHRACH H ET AL: "ROBOTICS, COMPUTING, AND BIOLOGY. AN INTERDISCIPLINARY APPROACH TO THE ANALYSIS OF COMPLEX GENOMES" INTERDISCIPLINARY SCIENCE REVIEWS,GB,HEYDEN, LONDON, vol. 22, no. 1, 1997, pages 37-44, XP000863340 ISSN: 0308-0188 the whole document ---	
A	LUEKING A ET AL: "Protein microarrays for gene expression and antibody screening" ANALYTICAL BIOCHEMISTRY,ACADEMIC PRESS, SAN DIEGO, CA,US, vol. 270, 1999, pages 103-111, XP002127382 ISSN: 0003-2697 cited in the application the whole document --- -/--	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BÜSSOW ET AL: "A METHOD FOR GLOBAL PROTEIN EXPRESSION AND ANTIBODY SCREENING ON HIGH-DENSITY FILTERS OF AN ARRAYED cDNA LIBRARY" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 26, no. 21, November 1998 (1998-11), pages 5007-5008, XP002114084 ISSN: 0305-1048 cited in the application the whole document ---	
T	CHOI J -W ET AL: "A new magnetic bead-based, filterless bio-separator with planar electromagnet surfaces for integrated bio-detection systems" SENSORS AND ACTUATORS B,ELSEVIER SEQUOIA S.A., LAUSANNE,CH, vol. 68, no. 1-3, 25 August 2000 (2000-08-25), pages 34-39, XP004216589 ISSN: 0925-4005 the whole document -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/06271

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9957311 A	11-11-1999	AU 4136999 A	23-11-1999
DE 19854003 A	25-05-2000	GB 2343949 A	24-05-2000

PATENT COOPERATION TREATY

PCT

REC'D 19 OCT 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
D 1920 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/06271	04/07/2000	05/07/1999
International Patent Classification (IPC) or national classification and IPC C12N15/10		
Applicant MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER ...		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24/01/2001	17.10.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Valcarcel, R Telephone No. +49 89 2399 2368

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/06271

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-24 as originally filed

Claims, No.:

1-18 as received on 24/09/2001 with letter of 24/09/2001

Drawings, sheets:

1/6-6/6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
see separate sheet

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18
	No:	Claims	NONE
Inventive step (IS)	Yes:	Claims	NONE
	No:	Claims	1-18

Industrial applicability (IA) Yes: Claims 1-18
No: Claims NONE

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

The amendments filed with the letter dated 24.09.2001 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendment concerned is the one introduced in claim 1 (d): "wherein steps (a), (b) and (c) are carried out **in parallel**. On page 5 of the description it is stated that library clones can be handled in parallel. The IPEA considers that this is not sufficient basis for the amended claim 1(d), since it is not disclosed that the steps (a), (b) and (c) are to be carried out in parallel. Nowhere else in the application as filed was found a basis for the cited amendment. Thus, the cited amendment has not been considered in this report and originally filed claim 1(d) has been considered instead.

Re Item IV

The present application lacks unity contravening Rule 13 PCT. The IPEA cannot find any "special technical feature" (in the sense of Rule 13.2 PCT) which links the different methods referred to in claims 1 to 18 of the present application. However, the IPEA elected to carry out examination for the subject-matter of all claims.

Re Item V

1. The document numbering corresponds to the order of citation in the search report.

2. The following documents (D) were not cited in the international search report.
   D10: DE 29614623 U
   D11: WO 9325912 A

3. The present application does not meet the requirements of Article 33.3 PCT since **the subject-matter of claim 1 does not involve an inventive step.**

   D3 and **D4** disclose methods for selection of at least one molecule able to interact with a given molecule comprising the steps of:
   (a) contacting a first molecule with a second molecule affixed to a magnetic particle (in D3 the heat-stable alkaline phosphatase, see abstract; and in D4 anti-IL-8, see first page, third column) under conditions allowing specific interaction:
   (b) subjecting the product of step (a) to at least one washing step (PBS in D3, see page 265, left column, second paragraph; Tris-buffered saline plus Tween 20 in D4, see second page, middle column);

(c) determining whether a specific interaction had occurred (see in D3 page 265, left column, second paragraph; and see second and third pages of D4);
(d) providing the molecules selected by steps (a) to (c) (the positive molecules were isolated in D3 and D4 for further analysis);
wherein steps (a) to (c) are carried in containers (microcentrifuge tubes or ELISA plates in D3; polypropylene centrifuge tubes in D4) using a magnetic particle separator (in D3 a magnetic separator from SIGMA, see page 264, right column, first paragraph; in D4 it is not specified which magnetic separator was used but it is assumed that one was used).

Thus, D3 and D4 disclose methods having all the technical features and technical effects referred to in claim 1, with the exception of the use of an **"automated device comprising a magnetic particle processor"**. The technical problem would have been to provide a method using an alternative magnetic separator. The solution of the present application is the use of an automated device comprising a magnetic particle processor. The solution does not involve an inventive step for the following reasons.

D10 and **D11** disclose automated devices comprising a magnetic particle processor. The person skilled in the art would have combined the teachings of either D3 or D4, with the teachings of D10 and D11 to arrive at the same solution as the one disclosed in claim 1 of the present application, since the advantages of using the devices of D10 or D11 in the methods of D3 and D4 are obvious. **Thus, the subject-matter of claim 1 does not involve an inventive step.**

It is noted that D3 focuses on the improvement of detection of a library of phage-displayed antibodies against one specific antigen (the heat-stable alkaline phosphatase). It is further noted that D4 compares the efficiency of biopanning phage display libraries by traditional plate vs. magnetic bead methods using a monoclonal antibody to human IL-8. However, the methods disclosed in D3 and D4 are applicable to a variety of different compounds. The fact that the experiments disclosed in D3 and D4 have been performed to screen display libraries by using either a phosphatase (D3) or an antibody (D4), does not mean that the skilled person would not contemplate to use also a variety of compounds (including a library of antigens, or a library of antibodies) to screen against the display libraries of D3 and

D4. The skilled person would contemplate this possibility taking into account the standard knowledge in the field, and the obvious advantage of screening simultaneously different compounds.

Furthermore, D4 discloses that the fact that biopanning using magnetic beads is more effective than plate biopanning has several practical consequences for investigators attempting to isolate phage capable of binding to biological targets (see page 214, middle column, second paragraph). Such biological targets comprise a vast variety of possible compounds. Thus, the IPEA considers that the skilled person would contemplate to use of automated devices (as the ones disclosed in D10 and D11) together with the methods of selection disclosed in either D3 or D4 to arrive at the same solution as the one proposed in claim 1 of the present application.

Furthermore, the skilled person would also contemplate to carry out the different steps using an array-based technology in view of the obvious advantage of screening simultaneously a plurality of compounds against a library of potentially interacting compounds.

4. Claims 2 to 18 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, and thus **the subject-matter of claims 2 to 18 does also not involve an inventive step.**

Re Item VI Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO 99 57311	11 November 1999	30 April 1999	30 April 1998
DE 198 54 003	25 May 2000	18 November 1998	18 Nov.1998

Re Item VIII

Claim 1 is unclear (and therefore also claims 2 to 18 which refer to it) since the expression **"specifically interacting molecules"** is vague and undefined rendering the scope of these claims unclear. Furthermore, the expression **"magnetic particl- proc-ssor"** is also unclear form the wording of the claim alone in contrast to the requirements of Article 6 PCT (see PCT Preliminary Examination Guidelines, Chapter III, Section 4.2).

REPLACED BY ART 34 AMDT

CLAIMS

1. A method for the selection of at least one member of a number of specifically interacting molecules, said method comprising as the first step involving the contact of said interacting molecules:

   (a) contacting a first molecule with a second molecule affixed to a magnetic particle under conditions that allow a specific interaction between said first and second molecule to occur;

   and further the steps of:

   (b) subjecting the product obtained in step (a) to at least one washing step;

   (c) determining whether a specific interaction between said first and second molecule had occurred; and, if said specific interaction had occurred,

   (d) providing said first and/or second molecule selected by steps (a) to (c),

   wherein steps (a), (b) and (c) are carried out in (a) container(s) preferably representing an arrayed form, e.g. in (a) microtiter plate(s), using an automated device comprising a magnetic particle processor.

2. The method of claim 1, wherein said first and/or second molecule is an organic molecule and/or a mixture of organic molecules and/or inorganic molecules.

3. The method of claim 1 or 2, wherein said first and/or second molecule is a hapten.

4. The method of claim 2 or 3, wherein said first and/or second molecule is a cDNA expression product, and/or a (poly)peptide, and/or a nucleic acid, and/or a lipid, and/or a sugar, and/or a steroid, and/or a hybrid of said molecules.

5. The method of claim 4, wherein said cDNA expression product is an antibody or a fragment or a derivative thereof, an enzyme or a fragment thereof, a surface protein or a fragment thereof, or a nucleic acid-binding protein or a fragment thereof.

6. The method of any one of claims 1 to 5, wherein said first molecule is a (poly)peptide presented on the surface of organisms (e.g. phage, viruses, bacteria, eukaryotic cells) and/or organelles (e.g. ribosome) and/or soluble molecules (e.g. nucleic acids, protein-nucleic acid hybrids) and wherein the method further comprises after step (b) and prior to step (c) the step of:
(b') amplifying a (poly)peptide specifically interacting with said second molecule, wherein step (b') is carried out in (a) container(s) preferably representing an arrayed form, e.g. in (a) microtiter plate(s).

7. The method of claim 6, wherein prior to step (a) said library of first molecules (library 1) is preabsorbed with unloaded magnetic particles and/or molecules competitive (cross-reactive) to second molecules (target, library 2).

8. The method of claim 6 or 7 which further comprises after step (c) and prior to step (d) the step of:
(c') repeating steps (a), (b) and (c) and, optionally, step (b') at least once.

9. The method of claim 8, wherein steps (c) and (c') are performed in parallel.

10. The method of any one of claims 1 to 9, wherein said number of specifically interacting molecules is a pair of interacting molecules.

11. The method of any one of claims 1 to 9, wherein said number of specifically interacting molecules are three or more interacting molecules.

12. The method of any one of claims 1 to 11 further comprising the step of characterizing said first and/or second molecule and/or the corresponding genetic information.

13. The method of any one of claims 1 to 12, wherein said second molecule target is affixed to said magnetic particle via an affinity tag (e.g. a metal-chelating tag, an epitope tag, an enzyme binding domain, calmodulin, biotin, Strep-tag, protein A, protein G or protein L) and/or unspecific adsorption (e.g. plastic surface) and/or covalent binding (e.g. via functional groups such as NH_2-, COOH-, SH-groups).

14. The method of claim 13, wherein said metal-chelating tag is a His-tag, and/or said epitope tag is an HA-tag, a c-myc-tag, a VSV-G-tag, an α-tubulin-tag, a B-tag, an E-tag, FLAG, a His-tag, an HSV-tag, a Pk-tag, a protein C-tag, a T7-tag, EpiTag™, a V5-tag or an S-tag, and/or said enzyme binding domain is cellulose binding domain, barnase or maltose binding protein.

15. The method of any one of claims 1 to 14, wherein step (c) is effected by immunological means.

16. The method of claim 15, wherein step (c) is effected by ELISA, RIA, western/colony blotting, FACS or immunohistochemistry.

17. The method of claim 15 or 16, wherein step (c) is effected in (micro-)array format, preferably on a membrane and/or filter and/or a glas slide and/or in a microtiter plate.

18. A method for the production of a pharmaceutical composition comprising the steps of the method of any one of claims 1 to 17 and further the step of formulating said first and/or second molecule selected and/or characterized by the method of any one of claims 1 to 17 or a functionally and/or structurally equivalent derivative thereof in a pharmaceutically acceptable form.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
D 1920 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 06271	04/07/2000	05/07/1999
Applicant		
MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER ...		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [ ] as suggested by the applicant.

   [X] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/06271

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 C12N15/62 G01N33/543 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, CAB Data, STRAND, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 57311 A (MAX PLANCK GESELLSCHAFT ;WALTER GERALD (DE); BUESSOW KONRAD (DE);) 11 November 1999 (1999-11-11) claims 1-27 ---	1-6,10, 12-18
P,X	DE 198 54 003 A (JENOPTIK JENA GMBH) 25 May 2000 (2000-05-25) the whole document ---	1,2
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 December 2000	22/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	KALA MRINALINI ET AL: "Magnetic bead enzyme-linked immunosorbent assay (ELISA) detects antigen-specific binding by phage-displayed scFv antibodies that are not detected with conventional ELISA." ANALYTICAL BIOCHEMISTRY, vol. 254, no. 2, 15 December 1997 (1997-12-15), pages 263-266, XP002154707 ISSN: 0003-2697 cited in the application the whole document ---	
A	MCCONNELL STEPHEN J ET AL: "Biopanning phage display libraries using magnetic beads vs. polystyrene plates." BIOTECHNIQUES, vol. 26, no. 2, February 1999 (1999-02), pages 208-214, XP002154708 ISSN: 0736-6205 cited in the application the whole document ---	
A	MAIER ET AL: "AUTOMATED ARRAY TECHNOLOGIES FOR GENE EXPRESSION PROFILING" DRUG DISCOVERY TODAY,GB,ELSEVIER SCIENCE LTD, vol. 2, no. 8, August 1997 (1997-08), pages 315-324, XP002103832 ISSN: 1359-6446 the whole document ---	
A	LEHRACH H ET AL: "ROBOTICS, COMPUTING, AND BIOLOGY. AN INTERDISCIPLINARY APPROACH TO THE ANALYSIS OF COMPLEX GENOMES" INTERDISCIPLINARY SCIENCE REVIEWS,GB,HEYDEN, LONDON, vol. 22, no. 1, 1997, pages 37-44, XP000863340 ISSN: 0308-0188 the whole document ---	
A	LUEKING A ET AL: "Protein microarrays for gene expression and antibody screening" ANALYTICAL BIOCHEMISTRY,ACADEMIC PRESS, SAN DIEGO, CA,US, vol. 270, 1999, pages 103-111, XP002127382 ISSN: 0003-2697 cited in the application the whole document --- -/--	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	BÜSSOW ET AL: "A METHOD FOR GLOBAL PROTEIN EXPRESSION AND ANTIBODY SCREENING ON HIGH-DENSITY FILTERS OF AN ARRAYED cDNA LIBRARY" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 26, no. 21, November 1998 (1998-11), pages 5007-5008, XP002114084 ISSN: 0305-1048 cited in the application the whole document ---	
T	CHOI J -W ET AL: "A new magnetic bead-based, filterless bio-separator with planar electromagnet surfaces for integrated bio-detection systems" SENSORS AND ACTUATORS B,ELSEVIER SEQUOIA S.A., LAUSANNE,CH, vol. 68, no. 1-3, 25 August 2000 (2000-08-25), pages 34-39, XP004216589 ISSN: 0925-4005 the whole document -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/06271

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9957311 A	11-11-1999	AU 4136999 A	23-11-1999
DE 19854003 A	25-05-2000	GB 2343949 A	24-05-2000